Karolinska Institutet

Institutionen för mikrobiologi, tumör-och cellbiologi

Regulatory networks of c-di-GMP signalling involved in biofilm formation motility and host pathogen interactions in Salmonella typhimurium

AKADEMISK AVHANDLING
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ABSTRACT

Adaptation to altered environmental conditions is one of the fundamental bacterial characteristics. The ubiquitous second messenger c-di-GMP plays a key role to adopt bacterial behaviour. Transition from motility to sessility and from acute infection to chronic infection is regulated by c-di-GMP signalling in many bacteria. C-di-GMP is synthesised by the diguanylate cyclase activity of GGDEF domains and hydrolysed by the phosphodiesterase activity of EAL or HD-GYP domains respectively.

In this thesis we investigated the role of individual GGDEF/EAL domain proteins encoded by the genome of *Salmonella typhimurium* UMR1 in regulation of key virulence phenotypes such as invasion into HT-29 epithelial cells, induction of a pro-inflammatory immune response and colonization of the gastrointestinal tract of streptomycin treated mice, a model of human gastroenteritis **(Paper I)**. We identified distinct panels of GGDEF/EAL proteins associated with the regulation of each virulence phenotype. We characterized the networks of corresponding GGDEF/EAL domain proteins regulating invasion and induction of Interleukin-8 secretion. Our results revealed that GGDEF/EAL domain proteins regulate invasion of *S. typhimurium* into HT-29 cells through biofilm dependent and independent pathways. The major biofilm regulator CsgD is involved in c-di-GMP dependent inhibition of invasion and pro-inflammatory response.

Interestingly, EAL domain proteins STM1344 and STM1697 behaved unconventional compared to phosphodiesterase EAL proteins with respect to regulation of motility, biofilm formation and the virulence phenotype invasion **(Paper II** and **Paper III)**. By using genetic and biochemical approaches, we demonstrate that STM1697 and STM1344 inhibit motility and invasion by binding the master regulator of flagella regulon $FlhD_4C_2$. Upon binding to $FlhD_4C_2$, STM1697 suppresses the functionality of $FlhD_4C_2$ to affect expression of downstream genes. Bioinformatic analysis revealed that the EAL domain proteins STM1344, STM1697 and STM3611 belong to a sub-family of stand-alone EAL domain proteins from diverse species of Enterobacteria, which putatively regulate motility and subsequently other phenotypes such as biofilm formation and invasion.

Interestingly, the unconventional EAL domain proteins STM1344, STM1697, STM3375 and the phosphodiesterase STM3611 are all regulated on the posttranscriptional level by the carbon storage regulator CsrA, a global RNA binding protein **(Paper IV)**.

CsgD is a major regulator of biofilm formation and virulence. We characterized the regulatory networks of c-di-GMP metabolizing proteins regulating CsgD expression **(Paper V)**. GGDEF/EAL domain proteins affect rdar morphotype formation and CsgD expression at multiple levels. The EAL phosphodiesterase STM4264 targets a global pool of c-di-GMP to inhibit rdar morphotype formation and CsgD expression whereas the GGDEF-EAL phosphodiesterase STM1703 is hypothesized to act locally to inhibit rdar morphotype formation and CsgD expression in *S. typhimurium*.

In conclusion, in this thesis, the c-di-GMP signalling networks of biofilm formation, virulence phenotypes and motility of *S. typhimurium* were significantly extended.

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